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Modulation of the human immune response by the non-toxic and non-pyrogenic adjuvant aluminium hydroxide: effect on antigen uptake and antigen presentation

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SUMMARY

The regulatory effects of an adjuvant (aluminium hydroxide) on the early phase of the immune response have been investigated. Adsorbing a soluble antigen (tetanus toxoid) to aluminium hydroxide led to a significant increase ($P < 0{\cdot}001$) in antigen-induced T-cell proliferation (macrophage–T-cell interaction, MTI) making aluminium hydroxide-adsorbed antigens especially suitable to study immunoregulatory changes in the early phase of the immune response. First studies revealed that this increase was due to an enhancement of antigen uptake by the antigen-presenting cell. However, under conditions allowing for the uptake of comparable amounts of soluble (TT_S) or aluminium hydroxide-absorbed (TT_{AL}) antigen, T-cell proliferation in response to TT_{AL} was still higher than in response to TT_S. This difference was especially pronounced if suboptimal antigen concentrations were used and could be explained by differences in the TT_S- versus TT_{AL}-induced release of interleukin-1 (IL-1). Pulsing with TT_{AL} led to a substantial increase in IL-1 release by monocytes (MØ) which then subsequently augmented antigen-induced T-cell proliferation. This was further supported by addition of exogenous IL-1 to cultures of T cells and TT_S-pulsed MØs, which also significantly increased the T cells' proliferative response. These findings demonstrate that in the early phase of the immune response, aluminium hydroxide exerts its regulatory effect at the level of the antigen-presenting and mediator-releasing accessory cell.

Keywords adjuvanticity of aluminium hydroxide antigen uptake, influence of aluminium hydroxide IL-1 release antigen-specific T-cell proliferation T-cell reactivity to tetanus toxoid

INTRODUCTION

The efficacy of adjuvants is commonly judged by their influence on immunological functions, i.e. antibody production and/or resistance to tumours and parasites (Garnham & Humphrey, 1969; Herberman, 1974; Larsh & Weatherly, 1974; Leclerc, Audibert & Chedid, 1978; Sugimoto *et al.*, 1978; Yamamoto *et al.*, 1978; Bomford, 1980a, b; Mancino & Ovary, 1980). Little is known, however, about the way adjuvants exert their effects on the individual steps of the immune response. Yet knowledge of the adjuvants' interrelationship with immunoregulatory events is of utmost importance for the understanding of the adjuvants' mode of action as well as for the development of new adjuvants, which are urgently needed in human prophylaxis (WHO Technical Report Series, 1976). The aim of the study was to investigate functional aspects of adjuvant activity at the cellular

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level. Aluminium hydroxide, one of the most widely employed immunopotentiating substances, was used as a model system to examine the effect of an adjuvant on the response of human mononuclear cells to a commonly used antigen (tetanus toxoid). In this paper the influence of aluminium hydroxide on the early phases of the immune response—antigen uptake and antigen presentation—is discussed. We report an adjuvant-induced augmentation of macrophage–T-cell interaction (MTI) in response to tetanus toxoid. This augmentation was due to an increase in antigen uptake and interleukin-1 (IL-1) release by the antigen-presenting cells.

MATERIALS AND METHODS

Isolation and fractionation of mononuclear cells. Mononuclear cells (MNC) were isolated from the peripheral blood of tetanus toxoid-immune donors (last tetanus vaccination during the past 5 years) by buoyant density-gradient centrifugation on Lymphoprep (Nyegaard & Co., Oslo, Norway) (Bøyum, 1968). The cells from the interphase were aspirated, washed three times in saline and resuspended in RPMI 1640 medium supplemented with 15% pooled, heat-inactivated (30 min, 56°C) human AB serum, penicillin (100 iu/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM) (complete medium). After removal of adherent cells by multiple incubation steps in plastic petri dishes (1001, Falcon, Oxnard, CA), a T-enriched mononuclear cell population was prepared by E-rosetting and centrifugation on Lymphoprep as previously described (Eibl, Mannhalter & Ahmad, 1982). The T-enriched cell population (which will be referred to as T cells in this paper) contained 83–97% T cells (as judged by E-rosetting), 1–3% B cells (identified by determination of surface immunoglobulin positive cells) and less than 0·5% monocytes (MØ) (determined by non-specific esterase staining) (Koski, Poplack & Blaese, 1976).

Antigen. Liquid tetanus toxoid (TT_S, stock solution 2500 Lf/ml), aluminium hydroxide-adsorbed tetanus toxoid (TT_{AL}, stock solution 25 Lf/ml adsorbed to 2 mg/ml aluminium hydroxide) and aluminium hydroxide (AL, 2 mg/ml) were kindly provided by Immuno AG, Vienna, Austria.

Preparation of MØ monolayers and antigen pulsing. Monocytes were prepared by adherence to plastic surfaces. Mononuclear cells were suspended at a concentration of 1×10^7 cells/ml complete medium. 2 ml portions thereof were pipetted into plastic tissue culture plates (Macro Tray 635 TC, Greiner & Söhne, Kremsmünster, Austria) and incubated for 3 h at 37°C in a CO_2 incubator (5% CO_2 in humidified air). After aspiration of the non-adherent cell population, the MØ monolayers were washed three times with saline and incubated overnight in complete medium. These adherent cells, which were $87 \pm 7\%$ ($\bar{x} \pm$s.d.) esterase positive, were used as the source of antigen-presenting cells. The MØ monolayers were then pulsed in a CO_2 incubator at 37°C with TT_S, TT_{AL}, or AL at the concentrations and for the time periods indicated. If not stated otherwise, the antigen concentration used for pulsing was 5 Lf in 1 ml and the pulsing time was 3 h for TT_S and 1 h for TT_{AL} and AL. At the end of the pulsing step, the MØs were extensively washed with saline, gently scraped off with a rubber policeman, resuspended in complete medium and adjusted to the appropriate concentration (antigen-pulsed MØs). Control MØs were incubated without antigen and treated as described above.

Antigen-induced T-cell proliferation: assay conditions. T-cell proliferation in response to MØ-associated antigen was determined as previously described (Eibl, Mannhalter & Ahmad, 1982). Briefly, triplicate cultures containing 5×10^4 T cells and 1×10^4 autologous antigen-pulsed or control MØs in a volume of 0·2 ml complete medium were set up in flat-bottomed microtitre plates (Microtest II, Falcon) and incubated at 37°C in a CO_2 incubator for 7 days. If indicated, IL-1-containing supernatants prepared as described below by incubating MØ monolayers with AL were added at the beginning of the incubation period to give a final concentration of 25%. T-cell proliferation was determined by [^{3}H]-thymidine uptake. Incorporated radioactivity was measured by liquid scintillation counting (Mark III, Searle Analytic Inc., Des Plaines, IL). Results are expressed as mean dpm $\pm$ s.e.m.

Antigen uptake studies. Tetanus toxoid was labelled with ^{125}I by the lactoperoxidase-glucose oxidase method (Marchalonis, 1969). This iodination procedure did not alter the toxoid's binding characteristics to MØs, as could be demonstrated by competition assays with unlabelled tetanus

toxoid (data not shown). 0·4 Lf ($1·5 \times 10^6$ cpm) of ^{125}I-labelled tetanus toxoid were then mixed with cold tetanus toxoid to give a total of 20 Lf of TT_S or TT_{AL} in 1 ml of complete medium. Then MØ monolayers were pulsed with these antigen preparations for the time periods indicated. After removal of free antigen by extensive washing with saline, the MØs were gently scraped off with a rubber policeman and their number was determined with a Coulter counter.

Antigen uptake was assessed by measuring the amount of MØ-associated radoactivity with a γ-counter (Model 1195, Searle). Results, expressed as Lf of tetanus toxoid associated with 1×10^6 MØs, were calculated according to the following formula:

$$\text{Lf MØ-associated} = \frac{20}{\text{cpm added}} \times \text{cpm associated with } 1 \times 10^6 \text{ MØ.}$$

All experiments were performed in triplicate. The variations in MØ-associated radioactivity were less than 10% within each triplicate.

Stimulation of IL-1 release by human MØs and assay of IL-1 activity. Monocyte monolayers were prepared as described above. Immediately after removal of the non-adherent cells, TT_S (10 and 100 Lf), TT_{AL} (10 Lf) and AL (0·8 mg) in 1 ml RPMI 1640 medium supplemented with 5% pooled, heat-inactivated human AB serum, L-glutamine and antibiotics were added to the MØs. After a 24-h incubation period at 37°C in a CO_2 incubator, the culture supernatants were harvested and assayed for IL-1 activity (IL-1 sups.).

IL-1 activity was determined by the mitogenic effect of IL-1 on mouse (BALB/c, 4 weeks old) thymocytes (Gery, Gershon & Waksman, 1972; Lachman, 1983). Triplicate cultures of mouse thymocytes containing $1·5 \times 10^6$ cells and 10% or 1% of MØ culture supernatant in a total volume of 0·2 ml were set up in flat-bottomed microtitre plates (Microtest II, Falcon). PHA (1 μg/ml) was added if indicated and the cultures were kept at 37°C in a CO_2 incubator for 72 h. Thymocyte proliferation was determined by [^{3}H] thymidine uptake. For control experiments MØ monolayers were incubated with 1 ml of medium without antigen and the IL-1 activity of this supernatant was determined as described above.

Results expressed as mean Δ dpm $\pm$ s.e.m. were calculated according to the following formula:

Δ dpm = dpm obtained with IL-1 sups. − dpm obtained with medium containing the same concentrations of the substances used to induce IL-1 release.

RESULTS

MTI in response to TT_S and TT_{AL}

Coculturing T cells and autologous MØs previously pulsed with the same concentrations of TT_S and TT_{AL} (5 Lf in 1 ml) led to a marked difference in antigen-induced T-cell proliferation (Fig. 1). The proliferative response to TT_{AL} was significantly ($P < 0·001$, Student's t-test) higher than the proliferation induced by TT_S. In a total of 20 experiments the mean value $\pm$ standard error ($\bar{x} \pm$ s.e.m.) of the T-cell response to TT_S was $5·9 \pm 1·6 \times 10^3$ dpm as compared to $38·7 \pm 8·0 \times 10^3$ dpm in response to TT_{AL}. Pulsing the MØs with aluminium hydroxide alone in the same concentration as in TT_{AL} gave T-cell proliferations around background levels ($\bar{x}$ dpm $\pm$ s.e.m.: MØs pulsed with aluminium hydroxide $0·45 \pm 0·05 \times 10^3$; unpulsed control MØs $0·37 \pm 0·1 \times 10^3$).

Antigen uptake and MTI as a function of pulsing time

If the same amount of antigen (10 Lf in 1 ml) was used for pulsing, TT_{AL} was taken up to a much greater extent than TT_S (Fig. 2, MØ-associated antigen). Maximal uptake of TT_{AL} occurred after 30–60 min of pulsing and was at any given time about five to ten times higher than the uptake of TT_S, which reached a plateau between 1 and 3 h and did not increase if the pulsing was continued for up to 24 h (data not shown). Figure 2 (MTI) also shows that T-cell proliferation in response to TT_{AL} parallelled antigen uptake and reached its maximum after 1 h of pulsing. For TT_S however, MTI increased with increasing pulsing time, although the amount of MØ-associated antigen remained constant after the first hour of pulsing.

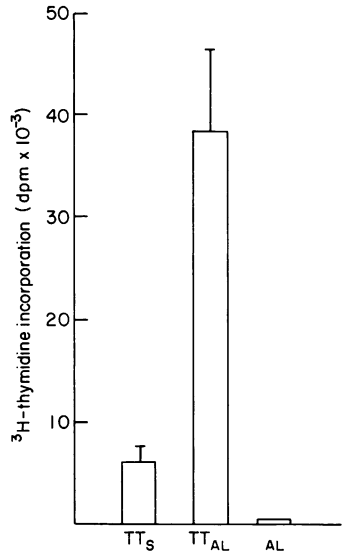

Fig. 1. Macrophage–T-cell interaction in response to soluble or aluminium hydroxide-adsorbed tetanus toxoid. MØs were pulsed with 5 Lf of soluble (TT_S) or aluminium hydroxide-adsorbed (TT_{AL}) tetanus toxoid, or with aluminium hydroxide alone (AL) in the same concentration as in TT_{AL} (0·4 mg/ml). Pulsed MØs and autologous T cells were cocultured in the absence of free antigen for 7 days. MTI was determined by antigen-induced T-cell proliferation and measured by [^{3}H]-thymidine uptake. Each bar represents mean dpm ± s.e.m. of 20 individual experiments. [^{3}H]-thymidine incorporation of T cells cocultured with unpulsed (control) MØs was less than 500 dpm.

Correlation of antigen uptake and antigen-induced T-cell proliferation

The data from Fig. 3 show that the differences in the T-cell response to TT_S and TT_{AL} were not only due to differences in antigen uptake. Experimental conditions were designed that allowed for the upake of equal amounts of TT_S and TT_{AL}, and antigen-induced T-cell proliferation was compared with the amount of MØ-associated antigen. Despite the presence of the same amount of MØ-associated antigen, T cells proliferated more strongly in response to TT_{AL}-pulsed MØs than in response to TT_S-pulsed MØs. This difference was especially pronounced when low amounts of antigen were taken up during the antigen-pulsing step.

Induction of IL-1 release by TT_S and TT_{AL}

As can be seen in Table 1, MØ monolayers stimulated with TT_{AL} released IL-1 to a greater extent than MØs stimulated with TT_S. If the direct mitogenicity of human IL-1 on mouse thymocytes was measured, supernatants derived from TT_S-stimulated MØ monolayers were almost devoid of IL-1 activity. The more sensitive PHA costimulation assay, however, revealed the presence of IL-1 in these supernatants, but in concentrations considerably lower than those induced by TT_{AL}. The results from Table 1 also show that induction of IL-1 release by TT_{AL} was largely due to the adjuvant. Aluminium hydroxide alone, if used in the same concentration as in TT_{AL}, induced the release of IL-1 activities comparable to TT_{AL}. Furthermore, higher TT_S concentrations (100 Lf/ml) led only to a slight increase of IL-1 in the culture supernatant.

The aluminium hydroxide-induced increase in IL-1 release could not be attributed to IL-1 released into the culture supernatant by MØs possibly killed by the relatively large amount of aluminium hydroxide applied. Neither pulsing with TT_{AL} nor pulsing with AL (at any of the concentrations used in this study) was found to be cytotoxic (judged by trypan blue exclusion, data not shown).

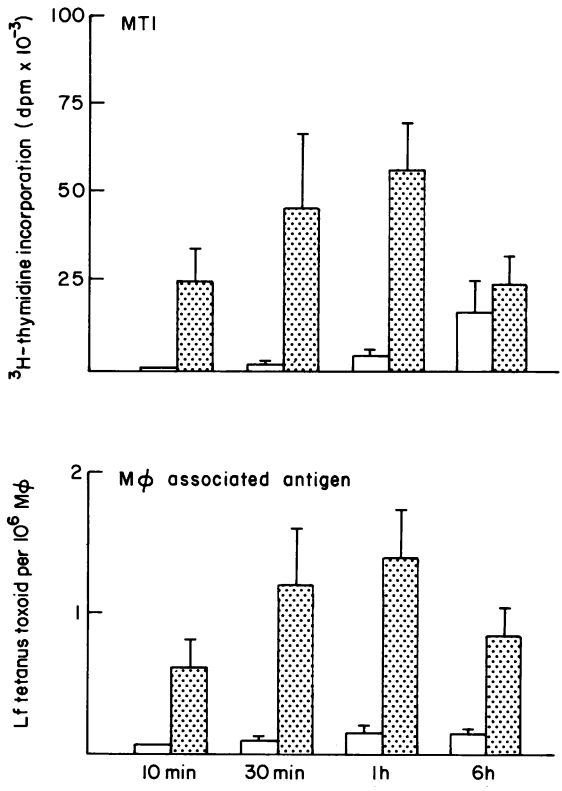

Fig. 2. Antigen uptake and macrophage–T-cell interaction as a function of pulsing time. To determine MTI, MØs were pulsed with the antigen (TT_S □, or TT_{AL} ▨; 10 Lf) for the time periods indicated and cocultured with autologous T cells for 7 days. T-cell proliferation was measured by [^{3}H] thymidine incorporation. Results are expressed as mean ± s.e.m. of three individual experiments. Antigen uptake was assayed by pulsing the MØs with ^{125}I-labelled antigen (TT_S □, or TT_{AL} ▨, 10 Lf) for the time periods indicated. Then the amount of MØ-associated radioactivity was determined and expressed as Lf tetanus toxoid associated with 10^6 MØ.

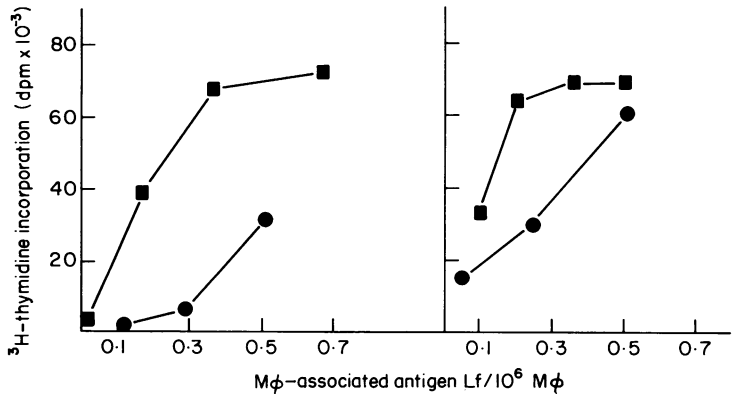

Fig. 3. Comparable amounts of MØ-associated tetanus toxoid induced a higher T-cell response if the antigen was adsorbed to aluminium hydroxide. MØ monolayers were pulsed with soluble (TT_S) or aluminium hydroxide-adsorbed (TT_{AL}) tetanus toxoid as described in the legends to Figs 1 and 2. Then the amount of MØ-associated antigen was correlated to antigen-induced T-cell proliferation. ■, MØ pulsed with TT_{AL}; ●, MØ pulsed with TT_S. The figure shows two representative experiments.

Table 1. Effect of TT_S, TT_{AL} and AL on IL-1 release by human MØs

Stimulant for IL-1 production	MØ culture supernatant in mouse thymocyte cultures (%)	Thymocyte proliferation $\Delta dpm \times 10^{-3} \pm$ s.e.m. PHA +	PHA −
—	10	1·1±0·6	0·1±0·04
	1	1·9±1·2	0·09±0·04
TT_S 100 Lf	10	6·2[a]	0·9[a]
	1	6·1[a]	1·8[a]
TT_S 10 Lf	10	3·4±2·0	0·8±0·6
	1	2·2±0·8	0·4±0·3
TT_{AL} 10 Lf	10	23·9±3·1	5·0±0·8
	1	14·2±3·0	3·7±1·2
AL (0·8 mg/ml)	10	30·2±2·8	6·8±0·8
	1	21·9±3·2	4·8±1·3

[a] Mean of two experiments.

MØ monolayers were incubated with medium, TT_S (10 and 100 Lf in 1 ml), TT_{AL} (10 Lf in 1 ml) and AL (1 ml, 0·8 mg/ml) for 24 h. IL-1 activity was determined by assaying the mitogenic effect of the MØ culture supernatant on mouse thymocytes in the presence and absence of PHA. The results represent mean values ± s.e.m. of six individual experiments.

Increase in TT_S-induced T-cell proliferation by addition of exogenous IL-1

The results presented in Table 2 provide evidence for the immunopotentiating effect of aluminium hydroxide via IL-1 release. Addition of exogenous IL-1 to cultures of T cells and TT_S-pulsed MØs led to a significant increase in antigen-induced T-cell proliferation if compared to cultures incubated in its absence. In the absence of antigen, IL-1 was not stimulatory and gave T-cell proliferations around background levels.

Table 2. Effect of exogenous IL-1 on TT_S-induced MTI

Experiment no.	MTI ($dpm \times 10^{-3}$) in response to TT_S-pulsed MØs −IL-1	TT_S-pulsed MØs +IL-1	Control MØs −IL-1	Control MØs +IL-1
1	11·6±1·3	24·5±9·4	0·3±0·1	0·5±0·2
2	15·2±0·1	27·7±5·3	0·9±0·2	1·1±0·2
3	3·3±0·9	13·2±1·7	0·3±0·1	0·5±0·2
4	5·4±1·9	16·6±2·9	0·9±0·1	0·8±0·2

MØ monolayers were pulsed with TT_S (10 Lf) and cocultured with autologous T cells in the presence and absence of IL-1-containing culture supernatant (25% final concentration). IL-1-containing culture supernatant was prepared by incubating MØ monolayers derived from 2×10^7 MNC with AL (0·8 mg/ml) for 24 h.

DISCUSSION

Uptake and processing of antigen by accessory cells is considered to be an important step for the initiation of the immune response (Rosenthal, 1980; Unanue, 1981). While the intracellular events leading to antigen catabolism have been widely dealt with (Elsbach, 1980; Griffin, 1982), little information is available on the metabolic processes resulting in re-expression of the antigen in the membrane of the phagocytic cell. This is especially true for the interrelationship between antigen processing, antigen re-expression and the release of mediators.

In the present paper we report a marked increase in T-cell proliferation in response to tetanus toxoid presented by autologous MØs if the antigen used for pulsing was adsorbed to aluminium hydroxide. First studies examining antigen uptake revealed that adsorbing tetanus toxoid to aluminium hydroxide led to a much greater amount of antigen becoming MØ-associated during the pulsing step. In consequence of the increased antigen uptake, an augmentation of antigen-induced T-cell proliferation could be observed, thus indicating a relationship between the amount of MØ-associated antigen and MTI. This finding is in good agreement with earlier studies performed by us as well as by others showing that changes in the amount of antigen used for pulsing were parallelled by changes in the magnitude of MTI (Todd, Reinherz & Schlossman, 1980; Eibl, Mannhalter & Ahmad, 1982), as well as changes in the efficacy of pulsed MØs to function as immunoadsorbants for antigen-specific T cells (Mannhalter *et al.*, 1983).

Uptake of tetanus toxoid in the absence of the adjuvant was about ten times lower than in its presence and was, after up to 3 h of pulsing, accompanied by a correspondingly low antigen-induced T-cell proliferation. Increasing the pulsing time led to an augmented MTI, but not to larger amounts of antigen becoming MØ-associated, which indicated that the metabolic pathway responsible for antigen re-expression had its own kinetics. Studies of antigen catabolism showed that following antigen uptake the majority of the antigen was rapidly degraded and released into the culture supernatant (Unanue & Askonas, 1968; Mannhalter *et al.*, 1984). Only a small amount of processed antigen was re-expressed in the MØ membrane in the form suitable to induce MTI (Ziegler & Unanue, 1981; Malek & Shevach, 1982). This amount might well be increased by extending the pulsing time.

However, the increase in MTI observed after pulsing with antigen adsorbed to the adjuvant was not only due to augmented antigen uptake. Under conditions allowing for the uptake of comparable amounts of antigen, the T-cell response to tetanus toxoid adsorbed to aluminium hydroxide was still higher than to soluble tetanus toxoid. This difference was especially pronounced in response to low amounts of MØ-associated antigen. Under these conditions one of the signals required for MTI might be induced or amplified by the adjuvant.

The signals required to induce MTI comprise: (a) processed antigen in the membrane of the antigen-presenting cell, (b) Ia-like antigen, and (c) mediators produced and released by the antigen-presenting cell (e.g. IL-1) (Unanue, 1981; Mizel, 1982). Since neither pulsing with soluble nor pulsing with aluminium hydroxide-adsorbed antigen induced significant changes in the amount of surface Ia expressed by the antigen-presenting cells (data not shown), our interest focused on the production and release of mediators. It could be shown that the adjuvant aluminium hydroxide was a potent inducer of IL-1 release, as had also been demonstated by other investigators for various substances known to exert adjuvant activity (Mizel, Oppenheim & Rosenstreich, 1978; Togawa, Oppenheim & Mizel, 1979; Oppenheim *et al.*, 1980). Thus part of the aluminium hydroxide's adjuvanticity might be due to increased production of mediators, a finding that correlates well with recent reports in the literature demonstrating an immunoenhancing activity of IL-1 if administered *in vivo* shortly after the antigen (Staruch & Wood, 1983). Since IL-1 is known to have a marked effect on T-cell proliferation (Mizel, 1982), the increased amount of IL-1 available after pulsing with aluminium hydroxide-adsorbed antigen might also account for the augmentation of MTI. This was further supported by addition of exogenous IL-1 to cultures of T cells and TT_S-pulsed MØs, which significantly augmented antigen-induced T-cell proliferation. Although additional influences on TT_{AL}-induced T-cell proliferation by other mediators such as IL-2 (Palacios, 1982), antigen-specific inducer factor (Lawrence & Borkowsky, 1983), etc., have to be taken into account as well, the results presented in this paper strongly suggest that the adjuvant aluminium hydroxide exerts its

immunopotentiating effect mainly at the level of antigen presentation (by increasing and/or accelerating antigen uptake) and IL-1 release.

In contrast to bacterial adjuvants, aluminium hydroxide is free of side effects because of absent antigenicity, toxicity and pyrogenicity. This and the significant *in vitro* T-cell response observed with aluminium hydroxide adsorbed antigen under suboptimal conditions (low antigen concentrations, not recently immunized individuals) make combinations of this adjuvant with antigens especially suitable for studying immunoregulatory events of the early phase of the immune response. Using aluminium hydroxide-adsorbed tetanus toxoid we were able to detect immunoregulatory changes following tetanus booster immunization (Zlabinger *et al.*, 1984). These changes, which were expressed by a decrease of antigen-induced T-cell proliferation shortly after the booster, could not be observed with soluble tetanus toxoid due to low or absent pre-immunization responses. Thus, besides its long-known immunopotentiating effect *in vivo*, this adjuvant might, in combination with antigen, also facilitate studies of immunoregulation performed *in vitro*.

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REFERENCES

BOMFORD, R. (1980a) The comparative selectivity of adjuvants for humoral and cell-mediated immunity. I. Effect on the antibody response to bovine serum albumin and sheep red blood cells of Freund's incomplete and complete adjuvants, alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipeptide and saponin. *Clin. exp. Immunol.* **39,** 426.

BOMFORD, R. (1980b) The comparative selectivity of adjuvants for humoral and cell-mediated immunity. II. Effect on delayed-type hypersensitivity in the mouse and guinea pig, and cell-mediated immunity to tumour antigens in the mouse of Freund's incomplete and complete adjuvants, alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipeptide and saponin. *Clin. exp. Immunol.* **39,** 435.

BØYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21,** (Suppl. 97), 77.

EIBL, M., MANNHALTER, J.W. & AHMAD, R. (1982) Macrophage–lymphocyte interaction in response to a bacterial antigen (*E. coli*). *Clin. exp. Immunol.* **47,** 260.

ELSBACH, P. (1980) Degradation of microoorganisms by phagocytic cells. *Rev. Infect. Dis.* **2,** 106.

GARNHAM, P.C.C. & HUMPHREY, J.H. (1969) Problems in leishmaniasis related to immunology. *Curr. Top. Microbiol. Immunol.* **48,** 29.

GERY, I., GERSHON, R.K. & WAKSMAN, B.H. (1972) Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. *J. exp. Med.* **136,** 128.

GRIFFIN, F.M., JR. (1982) Mononuclear cell phagocytic mechanisms and host defense. In *Advances in Host Defense Mechanisms* (ed. by J. I. Gallin & A.S. Fauci) vol. 1, p. 31. Raven Press, New York.

HERBERMAN, R.B. (1974) Cell-mediated immunity to tumor cells. *Adv. Cancer Res.* **19,** 207.

KOSKI, R.R., POPLACK, D.G. & BLAESE, R.M. (1976) A non-specific esterase stain for the identification of monocytes and macrophages. In *In vitro Methods in Cell Mediated and Tumor Immunity* (ed. by B. R. Bloom & J. R. David) p. 359. Academic Press, New York.

LACHMAN, L.B. (1983) Human interleukin 1: purification and properties. *Federation Proc.* **42,** 2639.

LARSH, J.E. & WEATHERLY, N.F. (1974) Cell-mediated immunity in certain parasitic infections. *Curr. Top. Microbiol. Immunol.* **67,** 113.

LAWRENCE, H.S. & BORKOWSKY, W. (1983) A new basis for the immunoregulatory activities of transfer factor—an arcane dialect in the language of cells. *Cell. Immunol.* **82,** 102.

LECLERC, C., AUDIBERT, F. & CHEDID, L. (1978) Influence of a synthetic adjuvant (MDP) on qualitative and quantitative changes of serum globulins. *Immunology*, **35,** 963.

MALEK, T.R. & SHEVACH, E.M. (1982) Nature of the antigenic complex recognized by T lymphocytes. IX. Direct immunochemical demonstration of nominal antigen on the macrophage cell surface. *Eur. J. Immunol.* **12,** 825.

MANCINO, D. & OVARY, Z. (1980) Adjuvant effects of amorphous silica and of aluminium hydroxide on IgE and IgG_1 antibody production in different inbred mouse strains. *Int. Archs. Allergy appl. Immun.* **61,** 253.

MANNHALTER, J.W., ZLABINGER, G.J., AHMAD, R. & EIBL, M.M. (1983) Human T cell proliferation in respone to *E. coli* presented by autologous macrophages is antigen specific. *Clin. exp. Immunol.* **54,** 95.

MANNHALTER, J.W., SCHRAMM, W., ZLABINGER, G.J., PINZKER, H., AHMAD, R. & EIBL, M. (1984) Ein funktioneller Defekt in der Frühphase der Immunantwort bei Patienten mit Hämophilie A. In *14. Hämophilie-Symposium Hamburg* 1983 (ed. by G. Landbeck & R. Marx), in press. Schattauer Verlag, Stuttgart.

MARCHALONIS, J.J. (1969) An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochem. J.* **113,** 299.

MIZEL, S.B. (1982) Interleukin 1 and T cell activation. *Immunological Rev.* **63,** 51.

MIZEL, S.B., OPPENHEIM, J.J. & ROSENSTREICH, D.L. (1978) Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D_1. I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* **120,** 1497.

OPPENHEIM, J.J., TOGAWA, A., CHEDID, L. & MIZEL, S. (1980) Components of mycobacteria and muramyl dipeptide with adjuvant activity induce lymphocyte activating factor. *Cell. Immunol.* **50,** 71.

PALACIOS, R. (1982) Mechanism of T cell activation: role and functional relationship of HLA-DR antigens and interleukins. *Immunological Rev.* **63,** 73.

ROSENTHAL, A.S. (1980) Regulation of the immune response—role of the macrophage. *New Engl. J. Med.* **303,** 1153.

STARUCH, M.J. & WOOD, D.D. (1983) The adjuvanticity of inerleukin 1 *in vivo*. *J. Immunol.* **130,** 2191.

SUGIMOTO, M., GERMAIN, R.N., CHEDID, L. & BENACERRAF, B. (1978) Enhancement of carrier-specific helper T cell function by the synthetic adjuvant, N-acetyl muramyl-L-alanyl-D-isoglutamine (MDP). *J. Immunol.* **120,** 980.

TODD, R.F. III, REINHERZ, E.L. & SCHLOSSMAN, S.F. (1980) Human macrophage–lymphocyte interaction in proliferation to soluble antigen. I. Specific deletion of lymphocyte proliferative activity on macrophage monolayers. *Cell. Immunol.* **55,** 114.

TOGAWA, A., OPPENHEIM, J.J. & MIZEL, S.B. (1979) Characterization of lymphocyte activating factor (LAF) produced by human mononuclear cells: biochemical relatonship of high and low molecular weight forms of LAF. *J. Immunol.* **122,** 2112.

UNANUE, E.R. (1981) The regulatory role of macrophages in antigen stimulation. Part two: Symbiotic relationship between lymphocytes and macrophages. *Adv. Immunol.* **31,** 1.

UNANUE E.R. & ASKONAS, B.A. (1968) Persistence of immunogenicity of antigen after uptake by macrophages. *J. exp. Med.* **127,** 915.

WHO TECHNICAL REPORT SERIES NUMBER 959 (1976) Immunological Adjuvants.

YAMAMOTO, A., KONDO, S., KAMEYAMA, S. & MURATA, R. (1978) Studies on adjuvants for human prophylactics. I. Comparison of efficiencies of different adjuvants at various stages of immunization with tetanus and diphtheria toxoids. *Japan. J. Med. Sci. Biol.* **31,** 263.

ZIEGLER, K. & UNANUE, E.R. (1981) Identification of a macrophage antigen-processing event required for I-region-restricted antigen presentation to T lymphocytes. *J. Immunol.* **127,** 1869.

ZLABINGER, G.J., MANNHALTER, J.W., AHMAD, R. & EIBL, M.M. (1984) Reduced antigen induced proliferation and surface Ia expression of peripheral blood T cells following tetanus booster immunization. *Clin. Immunol. Immunopathol.* **34,** 254.